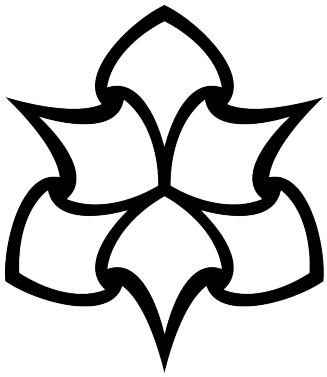

The potential for deep groundwater use by *Acacia papyrocarpa* (Western myall) in a water-limited environment

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Knowledge regarding the use of groundwater by plants has implications for successful mine rehabilitation and revegetation programs in water-limited environments. In this study we combined several approaches to investigate water sources used by *Acacia papyrocarpa* (Western myall) in the far west of South Australia, including stable isotope techniques and water potential measurements, analysis of groundwater and soil chemistry data, and root mapping techniques. Plant $\delta^{18}O$ signatures and water potentials were compared against a range of possible sources: rainwater, surface soil water (≤ 1 m depth) and deep groundwater (> 20 m depth). Our aim was to determine whether groundwater contributed to the mix of waters used by *A. papyrocarpa*.

Overall we found that trees sourced deep soil water rather than groundwater, although groundwater could not be dismissed entirely as a potential source. Root mapping data showed tree roots were capable of reaching groundwater at depths > 20 m, and isotope results indicated a potential contribution by groundwater to tree water use. However, low osmotic potentials and high acidity levels were shown to pose a likely barrier to water uptake, at least at the time of sampling. We conclude that because groundwater salinity and acidity is spatially variable in this region, plants with extensive root systems may be able to utilise zones of groundwater with lower salinity and pH levels. Overall this study contributes to our limited understanding of groundwater use by trees occurring in water-limited environments where groundwater is extremely deep (> 20 m depth).

KEY WORDS stable isotopes; tree water sources; water potential; rehabilitation

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INTRODUCTION

Mine sites in dry and remote regions of Australia are often established in areas considered high in conservation value. Some of the immediate impacts of mining include vegetation clearance and modifications to soil physical, chemical and biological properties (Jasper *et al.*, 1987; Rokich *et al.*, 2001), as well as changes to groundwater chemistry from tailings storage facilities (Wang *et al.*, 2014). In general, there are legislative requirements in place for mining companies to manage their environmental impacts during extraction processes, and to rehabilitate areas for the re-establishment of self-sustaining native ecosystems. The long-term success of revegetation programs requires an understanding of plant water use

strategies in undisturbed areas, so that conditions can, as far as possible, be optimised for re-establishing sustainable plant populations (Wang *et al.*, 2013).

Plant water use strategies, including seasonal shifts in groundwater dependency, have been studied in a range of ecosystems including montane coniferous forests (Xu *et al.*, 2011); karst systems (Swaffer *et al.*, 2014); and riparian systems (Holland *et al.*, 2006; Mensforth *et al.*, 1994; Thorburn *et al.*, 1993a; Wang *et al.*, 2013). Most research in Australia has focussed on semi-arid riparian ecosystems where groundwater is relatively shallow, < 5 m depth, and few studies have investigated water use by trees in regions where groundwater is more than 10 m deep. One exception is the study by Zencich *et al.* (2002), which used stable isotope techniques (deuterium δ^2H) to identify potential water sources for two species of *Banksia* growing over groundwater that ranged in depth from 2.5 m to 30 m. Both species were shown to use groundwater at shallow depths but not at its deepest, and the authors suggested that this pattern of water use was a function of moisture availability in shallower soil horizons, root distribution and maximum rooting depth.

The stable isotope oxygen-18 ($\delta^{18}O$) is also used to identify potential water sources used by plants. Two studies characterised $\delta^{18}O$ in deep soils of temperate semi-arid regions in Australia: Allison *et al.* (1983) and Allison *et al.* (1984) showed $\delta^{18}O$ signatures in soil water sampled below 0.5 m depth were negative values ranging between -2.0 and -4.0 ‰ relative to Standard Mean Ocean Water (SMOW). Soils were examined at intervals down to 15 m and 7 m depths respectively, and the results showed $\delta^{18}O$ signatures were relatively constant at depths below 3 m. In contrast, soil water above 0.5 m depth was found to have positive $\delta^{18}O$ values, most likely reflecting rainwater infiltration and enrichment from evaporation.

No significant fractionation of ^{18}O has been observed during plant uptake of soil water (Barbour, 2007) and thus, the isotopic composition of xylem water should match that of water sources (Mensforth *et al.*, 1994). However, isolating discrete sources is not always feasible because the isotopic composition of twig xylem water is generally a mixture of more than one source. The redistribution of water by tree roots can also produce complex water source patterns that are not necessarily discrete sources from a particular zone in the soil profile. As a consequence, multi-source mass balance analyses, such as IsoSource™ (United States Environmental Protection Agency), are used to estimate feasible proportional contributions for each possible water source, and have been used in several studies i.e. Fan *et al.* (2013), Wang *et al.* (2013) and Swaffer *et al.* (2013). The IsoSource™ model examines all possible combinations of each source contribution (0–100%) in small increments (e.g. 1-2%), and combinations that sum to the observed isotopic mixture within a small tolerance (e.g. < 0.1‰) are considered to be feasible solutions (Phillips *et al.*, 2003).

In addition to $\delta^{18}O$ measurements, water potential (Ψ) gradients are also used to infer the accessibility of water to plants. Soil Ψ helps to identify depths in the soil profile from which roots are physically capable of extracting water. It represents the sum of Ψs based on soil moisture (matric), soil salinity (osmotic) and gravity, measured in megapascals (MPa). Only soil regions with higher soil Ψs than shoot Ψs are available to a tree at any given time (Holland *et al.*, 2006). Shoot Ψs can be used as an indication of overall plant Ψ because water flow from roots to leaves is proportionate to a root–leaf potential difference and to a root–leaf hydraulic conductance term (Cook *et al.*, 2006). Water potentials from the saturated zone where matric potential approximates 0 MPa (i.e. groundwater), can also be compared using osmotic potentials calculated from the chloride concentration of the water (Holland *et al.*, 2006).

The survivorship of some plant species in arid ecosystems depends on their ability to access groundwater, which can be located at great depths i.e. > 20 m. Many tree species in arid regions are known to have roots that extend more than 50 m below the surface, e.g. *Boscia albitrunca* and *Acacia erioloba* (Jennings, 1974), and *Prosopis juliflora* (Phillips, 1963). Stone *et al.* (1991) present data on another 11 species, primarily forest trees, with roots that extend below 20 m depth.

In this paper we examine water use in the long-lived (250+ years) tree, *Acacia papyrocarpa* (Western myall), which has extensive lateral and vertical root systems. Vertical sinker roots branch from surface laterals that extend radially from the trunk to a distance > 20 m. Recent mining activity at the study area has uncovered vertical roots 22 m below the surface. This discovery highlights a discrepancy between the root-zone depth in undisturbed areas and the much shallower depth of overburden soils (6 - 8 m) replaced on top of tailings in post-mine rehabilitation sites. It raises questions about potential groundwater use, with groundwater frequently present at depths ranging between 20 and 50 m, and also about how altered plant-soil-water relations may affect the long-term survival of this species in rehabilitation sites. Shallow soil profiles due to insufficient overburden volumes is a widespread issue for mine rehabilitation across arid regions in Australia and elsewhere (Huang *et al.*, 2012). For many species, roots are required to grow in mine tailings (fine-grained waste material) which need to be physically and hydro-geochemically stable for plant growth. It is necessary to restore physical structures and hydraulic functions across the whole rooting zone and the complexity of this challenge often results in short-lived remediation success as soil structure and functions fail to develop, leading to poor plant survival and low recruitment levels (Huang *et al.*, 2012).

In this study we analysed $\delta^{18}O$ from xylem tissue collected from twigs, trunks, opposing lateral roots and taproots of *A. papyrocarpa,* as deep-rooted species with bimodal root architecture are likely to access water from a variety of sources. Xylem $\delta^{18}O$ signatures and shoot Ψs were compared against a range of possible sources: rainwater, surface soil water at four depths ≤ 1 m and deep groundwater > 20 m below surface. The overall aim of our research was to determine whether groundwater contributed to the mix of waters used by *A. papyrocarpa*.

METHODS

Study site

The study site (30°50'17.99"S and 132°12'10.37"E) was located at the Jacinth-Ambrosia (JA) mine site in Yellabinna Regional Reserve, approximately 200 km north-west of Ceduna in South Australia (Figure 1). The nearest weather station to our study site was located at Tarcoola, which is 220 km to the east and in similar vegetation to that found at the study site. Mean monthly minimum and maximum temperatures at Tarcoola are, respectively, 4°C and 18°C in July and 18°C and 35°C in January. Mean annual rainfall at Tarcoola is 174 mm (BOM, 2014). Rainfall is generally low and evenly spread during winter months, however, large summer rainfalls can produce floods and often occur during La Niña years (Facelli *et al.*, 2008). Rainfall was below average at the site in the 12 months leading up to this study, leaving surface soil horizons very dry.

Soils at the study site are deep calcareous sandy loams consisting of a thick layer of brown sandy loam (average 4 m) generally overlying a narrow layer of calcrete (Pratt, 2008). Non-calcareous red sandy loam extends beneath the calcrete to a depth of approximately 10 m, below which is white sand (Pratt, 2008). The physio-chemical characteristics of the brown and red sandy loam can vary and areas of pH 9 and above are generally associated

with the presence of calcium carbonate (Bean *et al.*, 2012). Groundwater at the mine is restricted to fractured rock aquifers, which are heterogeneous and may have dual-porosity characteristics where groundwater is stored in preferential pathways and/or the rock matrix (Bean *et al.*, 2012). Natural groundwater depth is generally between 20 to 50 m, and salinity levels can be as high as 68 dS/m (unpublished data).

Vegetation at the study site is sparse open woodland dominated by *A. papyrocarpa* (Fabaceae), with interspersed sandy rises and creeks where *A. papyrocarpa* and *Eucalyptus oleosa* (Myrtaceae) co-occur. *Acacia papyrocarpa* is a long-lived tree to 10 m high, often with multiple stems and a rounded canopy that spreads outwards with age. Individuals reach maturity after approximately 75 years and their lifespan exceeds 250 years (Ireland, 1997). The species is restricted to semi-arid and arid regions in southern Australia where they form sparse open woodlands that extend across a narrow band fringing the Nullarbor Plain (Johnson *et al.*, 2001). The understorey plant community is dominated by perennial chenopod shrubs and a suite of annual forbs and grasses that emerge from the soil seed bank following suitable rainfall and temperatures.

Tree sampling for stable isotope analysis

Three trees (Myall 1, 2 and 3) were selected at an undisturbed site 150 m south of the JA tailing storage facility. The maximum distance between trees was 150 m. The location was chosen because the trees were in close proximity to monitoring bores, with maximum distance to bores < 380 m. Trees were of similar life form (i.e. age) and one tree was sampled per day over three consecutive days in mid-June (i.e. early winter; see also Figure 2). Two primary opposing lateral roots (i.e. north and south facing) were identified at the base of each tree and exposed using shovels and trowels. North and south aspects were chosen because of potential differences in solar radiation conditions experienced by plant leaves and soils (Maren *et al.*, 2015). Primary laterals were generally large (approx. 20 cm diameter) and woody, often with a secondary lateral root of smaller diameter (approx. 5 cm) which was relatively smooth-barked. Opposing secondary laterals were located on two trees (Myall 1 & 2). All roots were sampled within 50 cm distance from the trunk and between 20 and 50 cm soil depths. Myall 1 and Myall 2 taproots were accessed with shovels and a small excavator. It was not possible to access the taproot of Myall 3.

A modified wad punch (Blackwoods, Regency Park, South Australia) and hammer were used to extract small cores of xylem tissue (approx. 1 cm^3) from root and trunk positions. Twenty-five cores were collected at each sample position and immediately immersed in kerosene in 150 mL glass jars sealed with metal lids and secured with electrical tape to prevent evaporation. Similarly, tree trunks were sampled using a wad punch on opposite sides of the tree, matching the aspect of lateral roots.

Twigs (approximately 15 mm diameter) were collected from north and south aspects of each tree canopy. Bark was removed from twigs and twig lengths (approximately 200 mm) were cut into 15 mm sections and immersed in kerosene as described above. Small secondary roots were also processed in this manner.

Potential water sources – groundwater, rainwater and soil water

We examined isotopic signatures and Ψs from rainwater, soil water and groundwater at the site (Figure 2). Rainwater was collected on the first morning of sampling. Total rainfall measured for the day was < 2 mm, recorded by an onsite weather station. Groundwater was examined from three monitoring bores: MBN01D (40 m depth); MBN01S (35 m depth); and IH18 (23 m depth). Water samples were obtained from monitoring bores approximately two weeks after trees and soils were sampled, following purging and bore

recovery of aquifers by a commercial provider (OTEK Practical Environmental Solutions, Adelaide, South Australia). The delay in sampling groundwater was considered acceptable given isotope signatures were unlikely to change within a two week period, and this was validated by similar values obtained in subsequent sampling done 14 months later. Groundwater and rainwater samples were collected in triplicate and stored in glass McCartney bottles (Microteknik, Haryana, India).

To collect soil samples, a 1.2 m deep trench was excavated approximately 5 m to the west of each tree (i.e. outside the canopy edge). Soil bulk density rings (258 cm^3) were used to collect samples from the freshly exposed face of each trench at 0.1, 0.3, 0.5 and 1.0 m depths. Soil samples were stored in 500 mL glass jars with metal lids and sealed with electrical tape to minimise evaporation.

Isotope analyses

Azeotropic distillation (Revesz *et al.*, 1990) was used to extract water from plant xylem tissue and soils. Analysis of $\delta\,^{18}O$ was conducted with mass spectrometry as per Thorburn et al. (1993b) and Brunel et al. (1997). All isotope extractions and analyses were carried out by a commercial provider (Isotope Analysis Service, CSIRO Land and Water, Waite, South Australia). IsoSource™ (US EPA) was used to determine bounds for the contributions of each source as per Phillips *et al.* (2003). Combinations of each source contribution were analysed at 1.5% increments and were considered feasible within a tolerance of 0.01‰.

EC and pH measurements

Additional groundwater, rainwater and soil samples were collected concurrently to measure electrical conductivity ($EC_{1:5}$) and pH_{water}. Groundwater EC and pH were measured by a commercial provider (OTEK Practical Environmental Solutions, Adelaide, South Australia). Rainwater and soil EC and pH were analysed with an ultrameter (Myron L Company 6PSI ultrameter II). Soil EC and pH was determined using the 1:5 soil/water method and converted to $ECe_{1:5}$ with a texture conversion factor as per Wetherby (2003).

Plant shoot, groundwater and soil water potentials

Pre-dawn shoot Ψs were measured on each sampling day using a Scholander pressure chamber (PMS Instrument Company, USA). Three replicate shoot samples (approximately 5 mm diameter) were obtained from north and south-facing aspects of the canopy and measured immediately following collection. Total means of shoot Ψs are presented for each tree in our results.

Additional soil samples were collected from each trench to measure soil Ψs at four depths: 0.1, 0.3, 0.5 and 1.0 m. Soil was collected in bulk density rings and placed into 300 mL glass jars with metal lids and sealed with electrical tape. Total soil Ψ was calculated by adding together matric (P_m), osmotic (P_o) and gravitational (P_g) pressure potentials. Matric potential was determined by the 'filter paper' technique (Greacen *et al.*, 1989). The relation $P_o = O.36 \times EC \times 10^3$ was used to calculate osmotic pressure of soil solutions from EC measurements as per Allison *et al.* (1954). Gravimetric water content (g g^{-1}) was calculated from wet and dry weights, with soil dried at 120°C for 24 hours. Groundwater osmotic potentials were calculated as per Holland (2002). Gravitational pressure (0.098 MPa m^{-1}) was added to both soil and groundwater Ψs as per Taiz *et al.* (2010).

Root and soil samples collected from the mine pit

Collections of root and soil samples were made opportunely throughout the mining process, from the wall and floor of the pit. Soil samples were collected from the immediate

vicinity of root samples and analysed for EC, ECe and pH as per method above. A differential GPS (Trimble 5800™ and TSC3 controller) was used to verify the position of each set of samples i.e. latitude (x), longitude (y) and elevation (z). The original surface z value was then used to calculate the depth of each sample set. Several roots were selected for DNA sequencing to identify the species. The internal transcribed spacer 2 (ITS2) was PCR-amplified using a plant-specific forward primer (ITS2P, Hugh Cross, unpublished data, contact L. Clarke for details) and ITS2 S3R (Chen *et al.*, 2010). PCR products were Sanger sequenced using standard protocols as per Clarke *et al.* (2012). Putative identifications for each consensus sequence were obtained by performing a local BLAST search against a reference DNA sequence database generated from plant voucher specimens from the study site.

RESULTS

Spatial variation in $\delta^{18}O$ signatures

We observed variation in isotope signatures between trees, tree parts and water sources (Figure 3). There was no significant difference between north and south twig signatures within trees. Mean (±SEM) north and south twig signatures were: -1.47 ‰ ± 0.13 (Myall 1); -0.84 ‰ ± 0.01 (Myall 2); and -0.69 ‰ ± 0.06 (Myall 3) (Table 1). Twig $\delta^{18}O$ values were similar to taproot signatures in Myall 1 and Myall 2 (the taproot was not sampled in Myall 3). Root signatures were generally negative with some exceptions, for instance the positive signature obtained from the north-facing secondary root of Myall 1, suggests this root was sourcing water differently from other roots. North-facing primary and secondary roots from Myall 2 and both opposing primary roots from Myall 3 also had positive values. Signatures from rainwater and surface soils ≤ 1 m deep were positive, ranging from 2.19 ‰ to 9.70 ‰, reflecting rainwater infiltration and enrichment from evaporation. Groundwater signatures were variable: +0.44 ‰ (MBN01D); -0.98 ‰ (MBN01S); and -1.93 ‰ (IH18). This variability between groundwater sources was also detected in subsequent analyses 14 months later: +0.39 ‰ (MBN01D); -2.09 ‰ (MBN01S); and -2.26 ‰ (IH18). The decrease in $\delta^{18}O$ at MBN01S is likely attributable to groundwater mixing as a result of mining activities at the site (S. Doudle, pers. com. 2013).

Surface soils ≤ 1 m deep and rainwater as possible sources

IsoSource™ results indicate that for all trees examined, the 25th and 75th percentiles for possible surface soil water use ranged between 0 and 5% for mean twig water sources, indicating little or no contribution from surface soil water at the time of sampling (Table 1). Water potentials showed surface soils were too dry for trees to extract water (Figure 3), as a consequence of low soil water contents and naturally high salinity levels (Table 2). In contrast, there was a relatively high contribution from soil water detected in the north-facing secondary root of Myall 1 (6-29% at 0.5 m and 8-32% at 1.0 m depth), suggesting the root was sourcing soil water at > 1 m depth with similar $\delta^{18}O$ signatures to shallower horizons and a higher soil moisture content. This demonstrates an advantage of analysing signatures from multiple positions within a tree, especially trees with extensive and deep root networks, when examining complex water source patterns that may not necessarily be detected in twig signatures alone.

Rainwater use was considered feasible for all trees examined, despite only 2 mm of rain falling on the first day of sampling. For all trees, the 25th and 75th percentiles for possible rainwater use was low, ranging between 1 and 9 % for mean twig water sources (Table 1). For Myall 1, percentiles were similarly low for possible rainwater use in all primary and secondary roots (excluding SR-N). However, percentiles from Myall 2 and Myall 3 were

higher in primary and secondary roots, ranging between 3 and 18%, which may reflect a delay in the uptake of rainwater by roots and subsequent transportation to twigs. However, we cannot dismiss the possibility that rainwater has a similar $\delta^{18}O$ signature to soil water from soil horizons > 1 m depth or to deep groundwater (e.g. MBN01D).

Groundwater as potential water sources

Overall our results are inconclusive with regards to groundwater use. Water potential and salinity results suggest that trees were probably unable to extract water from MBN01D, as it was too saline (Figure 3 and Table 2). This was reflected in IsoSource™ results from mean twig signatures, with 25th and 75th percentiles for possible MBN01D use ranging between 0 and 12% (Table 1). Water potential results indicate that trees were able to extract water from MBN01S, however IsoSource™ results from mean twig signatures are ambiguous, with percentiles ranging between 0 to 32% (Figure 3 and Table 2). Although Ψ results suggest that trees were unable to extract water from IH18 (Figure 3), IsoSource™ results present moderate to high percentiles for possible use, ranging between 48 and 87%. The low pH (3.3) in groundwater from both IH18 and MBN01S is a likely obstacle to tree water use (Table 2). Analyses from soil samples collected alongside plant roots in the mine pit show roots occurring in soils with pH as low as 4.2 (Figure 4); however, it is uncertain whether trees could use groundwater with pH as low as 3.3, as in IH18 and MBN01S.

DISCUSSION

Plants in water-limited environments with deep root systems regularly extract water from deep soil horizons and groundwater because of a lack of reliable shallow water sources (Wang *et al.*, 2013). Groundwater use in arid regions of Australia is often discounted due to depth (i.e. > 20 m), especially when rooting depths are unknown, and high groundwater salinity levels. Root samples collected from the JA mine pit revealed *A. papyrocarpa* roots in the vicinity of deep groundwater, prompting us to consider it as a potential water source. Our findings indicate that at the time of sampling, the use of water from deep soil horizons > 1 m depth, was more probable than groundwater. However, we suggest that deep groundwater use by *A. papyrocarpa* in different spatial and temporal settings is likely.

Rainwater, on the first day of sampling, contributed little to twig water mixtures and this reflects the low amount of rainfall received (< 2 mm). Water potential results showed that trees were not able to extract soil water from horizons ≤ 1 m deep, and this reflects the dry conditions in the shallow soil horizons at the study site. Thus it follows that the similarities observed between $\delta^{18}O$ signatures in primary and/or secondary roots from all three trees examined, and those signatures of shallow soils ≤ 1 m deep, suggest that trees were likely sourcing water from deeper soil horizons (i.e. below those sampled in this study) with higher soil moisture contents.

The role of hydraulic redistribution needs to be considered here, which is the passive movement of water through xylem pathways, from wetter (high-water potential) to drier (low water potential) regions in the soil. After rainfall, surface soil water is transported downwards into deeper soil layers where it enables the growth and survival of deep root networks. When surface soils become dry in summer or during periods of drought, water is transported upwards via hydraulic lift where it can be used to sustain surface roots. This strategy has been documented in deep-rooted species occurring in arid environments (Bleby et al., 2010).

Given the depths at which we have observed *A. papyrocarpa* roots, the redistribution of water into deeper soil layers likely plays a critical role in the tree's water use strategies. There is minimal infiltration of rainwater into deep soil horizons (i.e. > 1 m depth) at the study site, making the vertical redistribution of water through xylem pathways potentially important for this species, with the process certainly requiring further examination. A tree's dependence on water stored in deep soil horizons has implications for species re-establishment and long-term survival in post-mine areas, particularly when considering modified soils and tailings often have different water holding capacities and soil chemistries than those of pre-disturbed soils (Rokich *et al.*, 2001). Potential repercussions are reduced rooting depths and restrictions to roots accessing deeper water sources in rehabilitation sites, which may compromise the ability of plants to subsist through extended dry periods. This process also has important implications for landscape hydrology and potentially the spatial distribution of understory plant species that may rely on the redistribution of water towards the surface (Burgess *et al.*, 2001).

The potential use of groundwater by *A. papyrocarpa*, is strongly suggested by the relatively high percentiles for possible IH18 groundwater use obtained from mean twig signatures in all three trees examined, ranging between 48 and 87%. However, Ψ results showed that trees were not capable of extracting water from IH18. This discrepancy may be attributable to the timing of sampling, as the Ψ from IH18 groundwater fits within the range of predawn shoot measurements previously recorded for *A. papyrocarpa* at this site (unpublished data). Alternatively, it may indicate that trees were sourcing water from soil regions deeper than were sampled in this study. For example, we may expect that $\delta^{18}O$ signatures in deep soils, i.e. outside the range we sampled, may be negative values within the vicinity of -2.0 and -4.0 ‰, as per Allison *et al.* (1983) and Allison *et al.* (1984). If so, then we cannot discount that signatures from deeper soils may be similar to the groundwater value recorded from IH18, and this may account for the high percentiles generated from IsoSource™. The characterisation of $\delta^{18}O$ from deeper soil horizons is needed to confirm whether this is the case.

Although salinity levels were very high in groundwater at the study site, salt toxicity is not likely to be an obstacle to groundwater use by *A. papyrocarpa*. *Acacia* species are well known for their widespread occurrence on naturally saline soils in Australia (Craig *et al.*, 1990) and numerous studies have demonstrated high salt tolerance in many species (Aswathappa *et al.*, 1987; Craig *et al.*, 1990; Thomson, 1987). Soils at the study site are naturally saline, and analyses of soil samples collected from the mine pit show roots occur in soils with ECe as high as 55 dS/m (Figure 4). In glasshouse trials, Craig *et al.* (1990) demonstrated growth and survival of several *Acacia* species in soils irrigated with saline solution as high as EC 95 dS/m. Also, other non-*Acacia* species have been shown to use extremely saline groundwater, with several *Eucalyptus* species occurring on floodplains along the River Murray in South Australia using groundwater with EC levels up to 33 dS/m (Thorburn *et al.*, 1993a).

Our results suggest that low Ψs and/or low pH are the primary obstacles to groundwater use by *A. papyrocarpa*. However, previous work in arid riparian environments has shown that trees tolerate high soil and groundwater salinities by having low transpiration rates which reduces water use, and that they are generally able to extract water at very low osmotic potentials (Costelloe *et al.*, 2008). In addition, roots have been shown to occur in soils at the study site with pH as low as 4.2 (Figure 4), suggesting high acid tolerance. This is supported by work of Ashwath *et al.* (1995) who examined acid tolerance in *Acacia* species and found many of the 36 species examined were able to grow and fixing nitrogen in soils of 4.1 pH_{water} without adverse effects. The groundwater pH value (3.3) for both

IH18 and MBN01S groundwater, is still considerably lower than known plant thresholds, and thus further investigation is needed to establish acid tolerance levels for *A. papyrocarpa*.

Overall, we cannot rule out groundwater use from this study because soil salinity is spatially variable and this may enable plants with extensive root systems to utilise zones of groundwater with lower salinity (Craig *et al.*, 1990). Acidity too, varies between different groundwater sources. Previous studies show plants undergo seasonal shifts in water use in response to water availability, with many increasing their groundwater dependency when other sources are no longer available. Wang *et al.* (2013) examined five species including two trees, in a semi-arid ecosystem in China, and found all species were highly dependent on groundwater during the dry season but reduced their dependence during the wet season. Similar shifts in groundwater dependency have been reported in a range of studies (McCole *et al.*, 2007; Mensforth *et al.*, 1994; Xu *et al.*, 2011). Consequently, future experimental design for examining water use by *A. papyrocarpa* should consider seasonal changes in water use patterns through the inclusion of multiple sampling times.

CONCLUSIONS

Water from deep soil horizons was most probably the primary water source used by *A. papyrocarpa* trees in our study, although deep groundwater could not be discounted as a potential source under different spatial and temporal settings. Further research is needed to determine pH tolerance of *A. papyrocarpa* and to characterise $\delta^{18}O$ in soil horizons > 1m depth, in order to refine our understanding of water sources. Attention should also focus on potential shifts in groundwater use patterns, the role of hydraulic redistribution in water sourcing and incorporating other co-occurring deep-rooted species into analyses. Our research highlights the implications of plant water sourcing for re-establishing sustainable plant populations in disturbed areas where water is limited.

ACKNOWLEDGEMENTS

This research was funded by linkage project grants provided by the Australian Research Council (LP120200637 and LP0991985). Iluka Resources Ltd. also contributed funding and in-kind support. We appreciate the assistance given by Dr Cameron Grant (School of Agriculture, Food and Wine, University of Adelaide), Jennifer Young (Australian Centre for Ancient DNA, University of Adelaide), Shane Doudle, Con Miller and Kerry Saunders (Iluka Resources Ltd.).

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526 Table 1 IsoSource™ estimates of percentage water use for three *A. papyrocarpa* trees (Myall
527 1-3) showing 25th and 75th percentile ranges. The mean (±SEM) of north and south twig
528 signatures were used for analyses: -1.47 ‰ ± 0.13 (Myall 1); -0.84 ‰ ± 0.01 (Myall 2); and -
529 0.69 ‰ ± 0.06 (Myall 3). TR = trunk; PR = primary root; SR = secondary root; N = north
530 aspect; S = south aspect; MBNO1D, MBNO1S & IH18 = groundwater monitoring bores.
531 Data is missing for Myall 1 TR-S due to insufficient water extracted for analysis.

Tree/ position	$\delta^{18}O$	Percentage twig water use estimates (%)							
		Soil depth (m)				Groundwater			Rain-water
		0.1	0.3	0.5	1.0	MBN 01D	MBN 01S	IH18	
MYALL 1	**‰**	**+6.99**	**+4.81**	**+3.16**	**+3.37**	**+0.44**	**-0.98**	**-1.93**	**+2.19**
Twigs	**-1.47**	0-0	0-2	0-2	0-2	0-5	3-14	78-87	1-4
TR-N	**-0.94**	0-2	0-3	0-5	0-5	2-9	5-26	57-74	1-6
TR-S	**-**	-	-	-	-	-	-	-	-
PR-N	**-0.87**	0-2	0-3	0-5	0-5	2-11	6-27	54-72	1-7
PR-S	**-1.05**	0-2	0-3	0-3	0-3	2-9	5-23	62-77	1-6
SR-N	**+3.08**	0-3	20-42	6-29	8-32	2-9	2-8	3-7	3-17
SR-S	**-0.71**	0-3	0-3	0-5	0-5	2-12	6-30	48-68	3-7
Taproot	**-1.18**	0-2	0-2	0-3	0-3	2-8	5-20	68-80	1-6
MYALL 2	**‰**	**+7.30**	**+9.70**	**+5.72**	**+5.75**	**+0.44**	**-0.98**	**-1.93**	**+2.19**
Twigs	**-0.84**	0-3	0-2	0-3	0-3	2-11	6-29	54-74	3-7
TR-N	**+0.57**	2-6	0-5	2-8	2-8	5-23	9-38	18-45	4-15
TR-S	**+0.07**	0-5	0-3	0-6	0-6	5-20	9-39	27-54	3-12
PR-N	**+0.82**	0-3	2-6	2-9	2-9	6-27	9-36	14-39	4-18
PR-S	**-0.15**	0-5	0-3	0-5	0-5	3-18	9-39	32-59	3-12
SR-N	**+0.53**	2-6	0-5	2-8	2-8	5-23	9-39	20-47	4-15
SR-S	**-0.37**	0-3	0-3	0-5	0-5	3-15	8-36	38-63	3-10
Taproot	**-1.19**	0-2	0-2	0-2	0-2	2-8	5-21	68-81	1-6
MYALL 3	**‰**	**+8.46**	**+8.25**	**+5.78**	**+4.7**	**+0.44**	**-0.98**	**-1.93**	**+2.19**
Twigs	**-0.69**	0-2	0-3	0-3	0-5	2-12	6-32	48-69	3-9
TR-N	**-1.14**	0-2	0-2	0-2	0-3	2-9	5-21	65-80	1-6
TR-S	**+0.06**	0-5	0-5	0-6	2-6	3-20	9-39	27-54	3-12
PR-N	**+0.42**	0-5	0-5	2-8	2-8	5-23	9-39	21-48	4-15
PR-S	**+0.58**	0-5	2-6	2-8	2-9	5-24	9-38	18-44	4-16

532

533 Table 2 Salinity ($EC_{1:5}$) and pH_{water} of rainwater, groundwater (IH18, MBN01S & MBN01D)
534 and soil ≤ 1m depths (Myall 1, 2 & 3 soil pits), and soil texture, $ECe_{1:5}$ and gravimetric water
535 content (GWC). SCL = sandy clay loam, LSCL = light sandy clay loam, CL = clay loam.

Type	Sample	Depth (m)	$EC_{1:5}$ dS/m	pH (water)	Texture	$ECe_{1:5}$ dS/m	GWC %
Water	Rainwater	0.0	0.7	6.6	-	-	-
	IH18	23.0	59.0	3.3	-	-	-
	MBN01S	35.0	23.9	3.3	-	-	-
	MBN01D	40.0	68.2	6.1	-	-	-
Myall 1	Soil	0.1	0.3	8.7	SCL	2.9	2.7
	Soil	0.3	1.5	9.7	LSCL	14.6	5.6
	Soil	0.5	2.1	9.8	CL	20.1	7.1
	Soil	1.0	4.7	9.7	SCL	45.1	5.6
Myall 2	Soil	0.1	0.5	8.3	SCL	4.8	2.9
	Soil	0.3	1.0	9.7	SCL	9.5	4.3
	Soil	0.5	1.3	9.9	SCL	12.1	5.0
	Soil	1.0	0.6	10.1	LSCL	5.6	2.1
Myall 3	Soil	0.1	0.6	8.7	SCL	6.0	3.6
	Soil	0.3	1.5	9.6	SCL	14.1	6.2
	Soil	0.5	2.5	9.9	CL	24.2	9.9
	Soil	1.0	3.4	9.7	SCL	32.5	6.0

536

Figure 1 Location of the study site in Yellabinna Regional Reserve, approximately 200 km north-west of Ceduna in South Australia.

Figure 2 Schematic showing sampling positions for $\delta^{18}O$ analysis of *A. papyrocarpa* xylem water (TW = twig; TR = trunk; PR = primary root; SR = secondary root; N = north; S = south), soil water and groundwater (MBNO1D, MBNO1S & IH18 = groundwater monitoring bores). Three separate trees were sampled.

Figure 3 Water potential (MPa) and $\delta^{18}O$ (‰ relative to VSMOW) results for three *A. papyrocarpa* trees (Myall 1-3) and their potential water sources. Soil Ψs from 0.1 m depths are not shown because values were more negative than -8 MPa, beyond the capacities of plants to extract water. Dotted lines represent the best fit for twig/shoot values and possible water sources. The dotted circle for Myall 1 highlights the strongly positive $\delta^{18}O$ value for SR-N. TW = twig/shoot; TR = trunk; PR = primary root; SR = secondary root; N = north aspect; S = south aspect; IH18, MBNO1S & MBNO1D = groundwater monitoring bores. Symbols: crosses = xylem tissue; squares = soil water; and circles = groundwater and rainwater.

Figure 4 A summary of root and soil samples collected from the mine pit showing rooting depths and associated soil pH_{water} and $ECe_{1:5}$ measurements. Samples collected from the pit beneath sandy rises and creek lines (i.e. where *A. papyrocarpa* co-occurs with *E. oleosa*) are included here. Only a selection of roots have been identified through DNA analysis and the dotted circles highlight the maximum known rooting depths for *A. papyrocarpa* and *E. oleosa*.